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<110> OKUNO, KAZUAKI YABUTA, MASAYUKI <120> POLYPEPTIDE CLEAVAGE METHOD USING OMPT PROTEASE VARIANT <130> 47259-5001-00-US (223490) <140> 10/573,821 <141> 2006-03-28 <150> PCT/JP04/014704 <151> 2004-09-29 <150> JP 2003-342183 <151> 2003-09-30 <160> 41 <170> PatentIn version 3.5 <210> 1 <211> 184 <212> PRT <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Synthetic polypeptide Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp 10 15 Trp Glu Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro 35 40 45 Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe 50 60 Pro Ala Pro Glu Ala Val Pro Glu Ser Leu Leu Asp Leu Pro Glu Ala 65 70 75 80 Asp Thr Val Val Pro Asp Ser Ser Asn Trp Gln Met His Gly Tyr 85 90 95 Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro 100 105 110

Pro Phe Val Pro Thr Glu Pro His His His Pro Gly Gly Arg Gln 115 120 125

Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Gly 130 140

Ser Gly Ser Pro Tyr Arg His Pro Arg His Ala Glu Gly Thr Phe Thr 145 150 155 160

Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile 165 170 175

Ala Trp Leu Val Lys Gly Arg Gly

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<213> Artificial Sequence

<223> Description of Artificial Sequence: Synthetic polypeptide

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Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe 50 55 60

Pro Ala Pro Glu Ala Val Pro Glu Ser Leu Leu Asp Leu Pro Glu Ala 65 70 75 80

Asp Thr Val Val Val Pro Asp Ser Ser Asn Trp Gln Met His Gly Tyr 85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro 100 105 110

Pro Phe Val Pro Thr Glu Pro His His His Pro Gly Gly Arg Gln
115 120 125

Met His Ala Ala Ala Ala Ala Ala Ala Ala Arg Arg Ala Ala Ala 130 135 140

47259500.txt Ala Gly Ser Pro Tyr Arg His Pro Arg His Ala Glu Gly Thr Phe Thr 145 150 155 160 Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile 165 170 175

Ala Trp Leu Val Lys Gly Arg Gly

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Trp Glu Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro 20 25 30

Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe 50 55 60

Pro Ala Pro Glu Ala Val Pro Glu Ser Leu Leu Asp Leu Pro Glu Ala 65 70 75 80

Asp Thr Val Val Val Pro Asp Ser Ser Asn Trp Gln Met His Gly Tyr 85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro 100 105 110

Pro Phe Val Pro Thr Glu Pro His His His Pro Gly Gly Arg Gln 115 120 125

Met His Ala Ala Ala Ala Ala Ala Ala Ala Arg Arg Ala Arg Ala 130 135 140

Ala Gly Ser Pro Tyr Arg His Pro Arg His Ala Glu Gly Thr Phe Thr 145 150 160

Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Page 3

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Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe 50 60

Pro Ala Pro Glu Ala Val Pro Glu Ser Leu Leu Asp Leu Pro Glu Ala 65 70 75 80

Asp Thr Val Val Pro Asp Ser Ser Asn Trp Gln Met His Gly Tyr 85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro 100 105 110

Pro Phe Val Pro Thr Glu Pro His His His Pro Gly Gly Arg Gln 115 120 125

Met His Ala Ala Ala Ala Ala Ala Ala Arg Arg Arg Ala Arg Ala 130 135 140

Ala Gly Ser Pro Tyr Arg His Pro Arg His Ala Glu Gly Thr Phe Thr 145 150 155 160

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Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe 50 60

Pro Ala Pro Glu Ala Val Pro Glu Ser Leu Leu Asp Leu Pro Glu Ala 65 70 75 80

Asp Thr Val Val Val Pro Asp Ser Ser Asn Trp Gln Met His Gly Tyr 85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro

Pro Phe Val Pro Thr Glu Pro His His His Pro Gly Gly Arg Gln

Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Phe Val Pro Ile 130 135 140

Phe Thr Tyr Gly Glu Leu Gln Arg Met Gln Glu Lys Glu Arg Asn Lys 145 150 155 160

Gly Gln

<210> 6 <211> 165

<212> PRT <213> Artificial Sequence

<223> Description of Artificial Sequence: Synthetic Page 5

polypeptide

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Trp Glu Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro 20 25 30

Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe 50 60

Pro Ala Pro Glu Ala Val Pro Glu Ser Leu Leu Asp Leu Pro Glu Ala 65 70 75 80

Asp Thr Val Val Val Pro Asp Ser Ser Asn Trp Gln Met His Gly Tyr 85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro 100 105 110

Pro Phe Val Pro Thr Glu Pro His His His Pro Gly Gly Arg Gln 115 120 125

Met His Ala Ala Ala Ala Ala Ala Ala Arg Arg Arg Ala Arg Phe 130 135 140

Val Pro Ile Phe Thr Tyr Gly Glu Leu Gln Arg Met Gln Glu Lys Glu 145 150 150

Arg Asn Lys Gly Gln 165

Trp Glu Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro 20 25 30 Page 6

<210> 7 <211> 167

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Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe 50 55 60

Pro Ala Pro Glu Ala Val Pro Glu Ser Leu Leu Asp Leu Pro Glu Ala 65 70 75 80

Asp Thr Val Val Pro Asp Ser Ser Asn Trp Gln Met His Gly Tyr 85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro 100 105 110

Pro Phe Val Pro Thr Glu Pro His His His Pro Gly Gly Arg Gln 115 120 125

Met His Ala Ala Ala Ala Ala Ala Ala Arg Arg Arg Ala Arg Ser 130 135 140

Tyr Ser Met Glu His Phe Arg Trp Gly Lys Pro Val Gly Lys Lys Arg 145 150 155 160

Arg Pro Val Lys Val Tyr Pro 165

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Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe 50 60

Pro Ala Pro Glu Ala Val Pro Glu Ser Leu Leu Asp Leu Pro Glu Ala 65 70 75 80

Asp Thr Val Val Val Pro Asp Ser Ser Asn Trp Gln Met His Gly Tyr 85 90 95

Asp Ala Pro ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro

Pro Phe Val Pro Thr Glu Pro His His His Pro Gly Gly Arg Gln

Met His Ala Ala Ala Ala Ala Ala Ala Ala Arg Arg Arg Ala Arg Cys 130 135 140

Gly Asn Leu Ser Thr Cys Met Leu Gly Thr Tyr Thr Gln Asp Phe Asn 145 150 155 160

Lys Phe His Thr Phe Pro Gln Thr Ala Ile Gly Val Gly Ala Pro Gly

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<210> 10

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<210> 11

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1 5
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1 5
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Arg Asn Lys Gly Gln
35
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Val Tyr Leu Ala Glu Glu Gly Gly Arg Lys Val Ser Gln Leu Asp Trp 50 55 60

Lys Phe Asn Asn Ala Ala Ile Ile Lys Gly Ala Ile Asn Trp Asp Leu 65 70 75 80

Met Pro Gln Ile Ser Ile Gly Ala Ala Gly Trp Thr Thr Leu Gly Ser 85 90 95

Arg Gly Gly Asn Met Val Asp Gln Asp Trp Met Asp Ser Ser Asn Pro 100 105 110

Gly Thr Trp Thr Asp Glu Ser Arg His Pro Asp Thr Gln Leu Asn Tyr Page 16

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Glu Glu Gly Gly Arg Lys Val Ser Gln Leu Asp Trp Lys Phe Asn Asn 35 40 45 Ala Ala Ile Ile Lys Gly Ala Ile Asn Trp Asp Leu Met Pro Gln Ile 50 55 60 Ser Ile Gly Ala Ala Gly Trp Thr Thr Leu Gly Ser Arg Gly Gly Asn 65 70 75 80 Met Val Asp Gln Asp Trp Met Asp Ser Ser Asn Pro Gly Thr Trp Thr 85 90 95 Asp Glu Ser Arg His Pro Asp Thr Gln Leu Asn Tyr Ala Asn Glu Phe 100 105 110 Asp Leu Asn Ile Lys Gly Trp Leu Leu Asn Glu Pro Asn Tyr Arg Leu 115 120 125 Gly Leu Met Ala Gly Tyr Gln Glu Ser Arg Tyr Ser Phe Thr Ala Arg 130 135 Gly Gly Ser Tyr Ile Tyr Ser Ser Glu Glu Gly Phe Arg Asp Asp Ile 145 150 160 Gly Ser Phe Pro Asn Gly Glu Arg Ala Ile Gly Tyr Lys Gln Arg Phe 165 170 175 Lys Met Pro Tyr Ile Gly Leu Thr Gly Ser Tyr Arg Tyr Glu Asp Phe 180 185 190 Glu Leu Gly Gly Thr Phe Lys Tyr Ser Gly Trp Val Glu Ser Ser Asp 195 200 205 Asn Asp Glu His Tyr Asp Pro Gly Lys Arg Ile Thr Tyr Arg Ser Lys 210 215 220 Val Lys Asp Gln Asn Tyr Tyr Ser Val Ala Val Asn Ala Gly Tyr Tyr 225 230 235 240 Val Thr Pro Asn Ala Lys Val Tyr Val Glu Gly Ala Trp Asn Arg Val 245 250 255 Thr Asn Lys Lys Gly Asn Thr Ser Leu Tyr Asp His Asn Asn Asn Thr 260 265 270 Ser Asp Tyr Ser Lys Asn Gly Ala Gly Ile Glu Asn Tyr Asn Phe Ile Page 18

275

280

Thr Thr Ala Gly Leu Lys Tyr Thr Phe 290